ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1999, p. 1270–1273 Vol. 43, No. 5
0066-4804/99/$04.00+0

β-Lactamase Production and Antimicrobial Susceptibility of Oral Heterogeneous *Fusobacterium nucleatum* Populations in Young Children

E. KÖNÖNEN,[1,2]* A. KANERVO,[1] K. SALMINEN,[3] AND H. JOUSIMIES-SOMER[1]

Anaerobe Reference Laboratory, National Public Health Institute, Helsinki,[1] and Pfizer Finland, Espoo,[3] Finland, and Department of Medical Microbiology and Immunology, University of Aarhus, Aarhus, Denmark[2]

Received 4 August 1998/Returned for modification 23 November 1998/Accepted 12 February 1999

Oral *Fusobacterium nucleatum* populations from 20 young, healthy children were examined for β-lactamase production. Ten children (50%) harbored, altogether, 25 β-lactamase-positive *F. nucleatum* isolates that were identified as *F. nucleatum* subsp. *polymorphum*, *F. nucleatum* subsp. *nucleatum*, and *F. nucleatum* subsp. *vincentii* (J. L. Dzink, M. T. Sheenan, and S. S. Socransky, Int. J. Syst. Bacteriol. 40:74–78, 1990). In vitro susceptibility of these β-lactamase-producing and 26 non-β-lactamase-producing *F. nucleatum* isolates was tested with penicillin G, amoxicillin-clavulanic acid, tetracycline hydrochloride, metronidazole, trovafloxacin, and azithromycin. Except for penicillin G, the antimicrobials exhibited good activity against all *F. nucleatum* isolates.

Fusobacterium nucleatum is one of the most frequently found anaerobic species in the oral cavity in early childhood (11). It is also commonly found in various infections in oral and nonoral sites. Pediatric infections in which *F. nucleatum* is involved are located mainly in the upper respiratory tract and the head and neck (5), suggesting an oral source. *F. nucleatum* is a heterogeneous bacterial group; its division into several subspecies has been made on the basis of electrophoretic patterns of enzyme mobilities or whole-cell proteins and on the basis of DNA-DNA homology (6, 7). However, *F. nucleatum* subspecies cannot be separated from each other by conventional biochemical testing alone. Differences in pathogenic potential among *F. nucleatum* subspecies have been reported (8, 27), indicating that the various subspecies may exhibit differences in such characteristics as β-lactamase production. Except for one report of a β-lactamase-producing *Fusobacterium nucleatum* subsp. *polymorphum* strain from the blood of a seriously ill patient (9), no data on β-lactamase production by different *F. nucleatum* subspecies exist. The first reports of penicillin resistance due to β-lactamase production by *F. nucleatum* were published in the mid-1980s (1, 3, 24). The frequency of β-lactamase production by fusobacteria seems to be increasing (2). We have observed surprisingly high frequencies of β-lactamase production by several anaerobic, gram-negative species in oral sites, especially by pigmented *Prevotella* spp., in infants and young children (13, 14, 19). In the present study, our aim was to examine β-lactamase production among heterogeneous oral *F. nucleatum* populations isolated from young, healthy children. Secondly, by using cellular fatty acid (CFA) analysis for subspecies identification, we tried to determine if β-lactamase production is characteristic only of a certain subspecies. Finally, the activity of potential alternative antimicrobials for *F. nucleatum* was determined.

Altogether, 123 *F. nucleatum* isolates originated from young, healthy children (11) from whom at least three simultaneous oral isolates were available. The children (10 boys and 10 girls ranging in age from 2 to 3.4 years) had not received systemic antimicrobial treatment within at least 1 month preceding the specimen collection (Table 1). The clinical *F. nucleatum* isolates, with various colony morphologies, were indole positive and lipase negative, produced butyric acid as a major metabolic end product, and did not convert lactate to propionate. *F. nucleatum* subsp. *polymorphum* ATCC 10953^T, *Fusobacterium nucleatum* subsp. *nucleatum* ATCC 25586^T, *Fusobacterium nucleatum* subsp. *vincentii* ATCC 49256^T, *Fusobacterium nucleatum* subsp. *fusiforme* NCTC 11326^T, *Fusobacterium nucleatum* subsp. *animalis* NCTC 12276^T, *Fusobacterium periodonticum* ATCC 33693^T (a closely related strain) were used as reference strains. The bacterial isolates were maintained in vials containing 20% sterilized skim milk at −70°C until further testing. An automatic CFA analysis, based on capillary column gas-liquid chromatography designed by the Microbial Identification System (MIS) (MIDI, Newark, N.J.) and with the Moore Broth Library database (versions 3.8 and 3.9) as a reference, was used as previously described (15) to presumptively identify the clinical *F. nucleatum* isolates to the subspecies level. A dendrogram (cluster analysis) was constructed for β-lactamase-positive *F. nucleatum* isolates and all reference strains.

β-Lactamase production of all 123 clinical *F. nucleatum* isolates was examined by the qualitative chromogenic cephalosporin disk (AB Biodisk, Solna, Sweden) test (20). In vitro antimicrobial susceptibility to penicillin G, amoxicillin-clavulanic acid, tetracycline hydrochloride, metronidazole, trovafloxacin, and azithromycin was examined for all β-lactamase-positive isolates and the corresponding number of β-lactamase-negative isolates by using the National Committee for Clinical Laboratory Standards (NCCLS)-approved agar dilution method (18). MICs were determined in parallel on brucella blood agar and on fastidious anaerobe agar (FAA; Lab M Ltd., Bury, England), both supplemented with sheep blood, hemin, and vitamin K_1 (21). To aid the endpoint reading, a viability indicator dye, triphenyltetrazolium chloride (TTC) (21), was used for *F. nucleatum* isolates with hazy growth.

All *F. nucleatum* isolates produced major amounts of $C_{14:0}$, $C_{16:0}$, and $C_{16:1}$-*cis*-9. The identification provided by MIS was used for the presumptive identification of the clinical *F. nucleatum* isolates to the subspecies level. A dendrogram was constructed for the β-lactamase-positive *F. nucleatum* isolates and all reference strains (Fig. 1). Most of the isolates clustered

* Corresponding author. Mailing address: Anaerobe Reference Laboratory, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland. Phone: 358-9-47448248. Fax: 358-9-47448238. E-mail: Eija.Kononen@ktl.fi.

TABLE 1. Characteristics of 20 children and the distribution of β-lactamase-producing *F. nucleatum* subspecies among their oral *F. nucleatum* populations[a]

Child	Gender	Age (mo)	Time (mo) from the last antibiotic course preceding specimen collection	Total no. of *F. nucleatum* isolates tested	No. of β⁺ *F. nucleatum* isolates found	Subspecies identification of β⁺ *F. nucleatum* isolates by CFA (no. of isolates)
1	M	31	6	4	0	
2	M	33	NA[b]	4	1	*polymorphum*
3	F	29	12	6	1	*nucleatum*
4	M	29	6	3	1	*nucleatum*
5	F	26	1	4	2	*polymorphum* (2)
6	M	34	NA	7	0	
7	F	29	12	7	0	
8	M	34	4	4	0	
9	M	31	1	6	5	*polymorphum* (3), *vincentii* (2)
10	F	34	8	9	0	
11	M	35	2	9	1	*polymorphum*
12	F	35	6	10	1	*polymorphum*
13	F	34	6	5	3	*nucleatum* (3)
14	F	36	12	9	6	*polymorphum* (5), *nucleatum* (1)
15	F	36	5	8	0	
16	M	36	12	6	0	
17	M	41	7	6	0	
18	F	24	12	4	4	*polymorphum* (3), *nucleatum* (1)
19	F	31	3	7	0	
20	M	36	3	5	0	
Total				123	25	

[a] β⁺, β-lactamase-producing.
[b] NA, not applicable; child did not receive antibiotics.

together with the indicated *F. nucleatum* type strains, but some clinical isolates formed a subcluster that did not concisely conform to the pattern of any type strain. For the latter isolates, similarity indices were less than 0.3 (the highest possible match is 1.0) and/or the differences between the primary and secondary identification choices by MIS were less than 0.1.

Ten children (50%) harbored a total of 25 β-lactamase-positive *F. nucleatum* isolates. Both β-lactamase-positive and β-lactamase-negative *F. nucleatum* strains were simultaneously isolated from 9 of 10 children. According to MIS, 16 of the β-lactamase-positive isolates were identified as *F. nucleatum* subsp. *polymorphum*, 7 isolates were identified as *F. nucleatum* subsp. *nucleatum*, and 2 isolates were identified as *F. nucleatum* supsp. *vincentii*. The distribution of the β-lactamase-producing subspecies within the group of children studied is seen in Table 1. Activities of several antimicrobials against β-lactamase-positive *F. nucleatum* isolates showed similar patterns on both agar media; the MICs determined on brucella agar and

TABLE 2. In vitro activities of antimicrobial agents against β-lactamase-producing and non-β-lactamase-producing *F. nucleatum* isolates

Antimicrobial agent	Growth medium	MIC (μg/ml) for[a]:					
		β⁺ isolates (n = 25)			β⁻ isolates (n = 26)		
		Range	MIC_{50}[b]	MIC_{90}[c]	Range	MIC_{50}	MIC_{90}
Penicillin G	Bru	2–256	64	256			
	FAA	1–256	64	128	≤0.03–1	≤0.03	≤0.03
Amoxicillin-clavulanate	Bru	≤0.125–1	0.25	1			
	FAA	0.25–1	0.5	1	≤0.125–0.5	≤0.125	≤0.125
Tetracycline hydrochloride	Bru	≤0.125–1	0.5	1			
	FAA	≤0.125–1	1	1	≤0.125–1	0.25	1
Metronidazole	Bru	≤0.125–0.25	≤0.125	0.25			
	FAA	≤0.125–0.25	≤0.125	0.25	≤0.125–0.25	≤0.125	≤0.125
Trovafloxacin	Bru	≤0.125–1	0.5	1			
	FAA	≤0.125–0.5	0.5	0.5	0.25–0.5	0.5	0.5
Azithromycin	Bru	0.25–4	1	2			
	FAA	0.25–2	0.5	2	0.25–2	0.5	1

[a] β⁺, β-lactamase-producing; β⁻, non-β-lactamase-producing. Data for β⁻ isolates are incomplete due to poor growth on brucella agar (Bru).
[b] MIC at which 50% of the isolates are inhibited.
[c] MIC at which 90% of the isolates are inhibited.

FAA agreed with each other within 1 $\log_2$ dilution. However, β-lactamase-negative isolates frequently exhibited poor growth on brucella plates. Table 2 presents the in vitro activities of penicillin G, amoxicillin-clavulanic acid, tetracycline hydrochloride, metronidazole, trovafloxacin, and azithromycin against 25 β-lactamase-producing and 26 non-β-lactamase-producing *F. nucleatum* isolates. MICs for the β-lactamase-positive isolates ranged from intermediate susceptibility (one isolate; MICs of 1 and 2 μg/ml on FAA and brucella agar, respectively) to high resistance to penicillin G (MIC of 256 μg/ml). Except for two isolates from the same child for which the MIC was 1 μg/ml, β-lactamase-negative isolates exhibited high susceptibility to penicillin G (MIC $\leq$ 0.03 μg/ml). Amoxicillin-clavulanic acid, tetracycline hydrochloride, metronidazole, and trovafloxacin had good activity against all *F. nucleatum* isolates. Azithromycin also proved to be effective against *F. nucleatum*, as only one strain for which MICs were 2 and 4 μg/ml on brucella agar and on FAA, respectively, was found.

A surprisingly high frequency of β-lactamase production by oral *F. nucleatum* was found in these young, healthy children, as half of them harbored β-lactamase-producing isolates. No differences between children with and without β-lactamase-positive *F. nucleatum* isolates with respect to gender, age, or preceding antimicrobial treatment were observed. β-Lactamase production coincided well with penicillin resistance; the MICs of penicillin G on brucella agar varied from 2 to 256 μg/ml for β-lactamase-positive isolates compared with the low MICs of penicillin G for β-lactamase-negative isolates. Notably, as both β-lactamase-producing and non-β-lactamase-producing variants can be simultaneously present in the mouth, several isolates per sample should be tested to determine the true rate of β-lactamase production within a bacterial species. As with our previous experience with pigmented *Prevotella* species (13, 14), the multiple isolate testing may partly explain the high frequency of β-lactamase production by *F. nucleatum* in young children observed in the present study.

CFA analysis under standardized conditions has proved to be a useful tool for the taxonomic characterization of anaerobic gram-negative bacilli (16). Tunér et al. (23) have successfully used CFA analysis to separate different *Fusobacterium* species. However, in differentiating *F. nucleatum* subspecies, an improved and expanded database that recognizes the National Collection of Type Cultures strains of *F. nucleatum* is needed. Another problem arises from the vast heterogeneity among *F. nucleatum* populations. The reported overlapping of subspecies (7) has caused uncertainty about whether three or four human subspecies exist. More recently, even the validity of the division of *F. nucleatum* into subspecies has been questioned (17). In the present study, CFA analysis was performed to presumptively identify the β-lactamase-positive *F. nucleatum* isolates to the subspecies level. The majority of these isolates were identified as *F. nucleatum* subsp. *polymorphum*; however, some were also identified as *F. nucleatum* subsp. *nucleatum* and *F. nucleatum* subsp. *vincentii*, which in most cases clustered together with indicated type strains. Thus, β-lactamase production seems not to be confined to one subspecies only but is shared by several *F. nucleatum* subspecies.

Special efforts are needed to test fusobacteria for their antimicrobial susceptibilities. Even by using an NCCLS-approved agar dilution method (18) that allows the addition of blood to the culture medium as an appropriate growth supplement for anaerobic bacteria, the problem of poor or hazy growth arises. "Tailing" of growth occurs due to cell wall-defective variants of *Fusobacterium* species (10). To solve the problem with exact end-point determination, we used TTC, a viability indicator dye, as an indicator to recognize the demarcation zone of viable growth. TTC has been successfully used to minimize inconsistency in interpreting endpoints for *Bilophila wadsworthia* (22), a fastidious anaerobic species with tendency to hazy growth on antimicrobial-containing media, a phenomenon identical to that seen with fusobacteria. Brazier et al. (4) compared different culture media for their capacity to support the growth of fusobacteria and found that the enriched culture medium designed especially for anaerobes, FAA, promoted the growth of fusobacteria and reduced their "tailing." In the present study, we accordingly determined the susceptibilities in tandem by using the NCCLS-recommended supplemented brucella agar and FAA. Both culture media were associated with nearly identical MICs for β-lactamase-positive *F. nucleatum* isolates. However, we were repeatedly confronted with difficulties in determining MICs due to the poor growth of β-lactamase-negative isolates on brucella agar. According to the previous (4) as

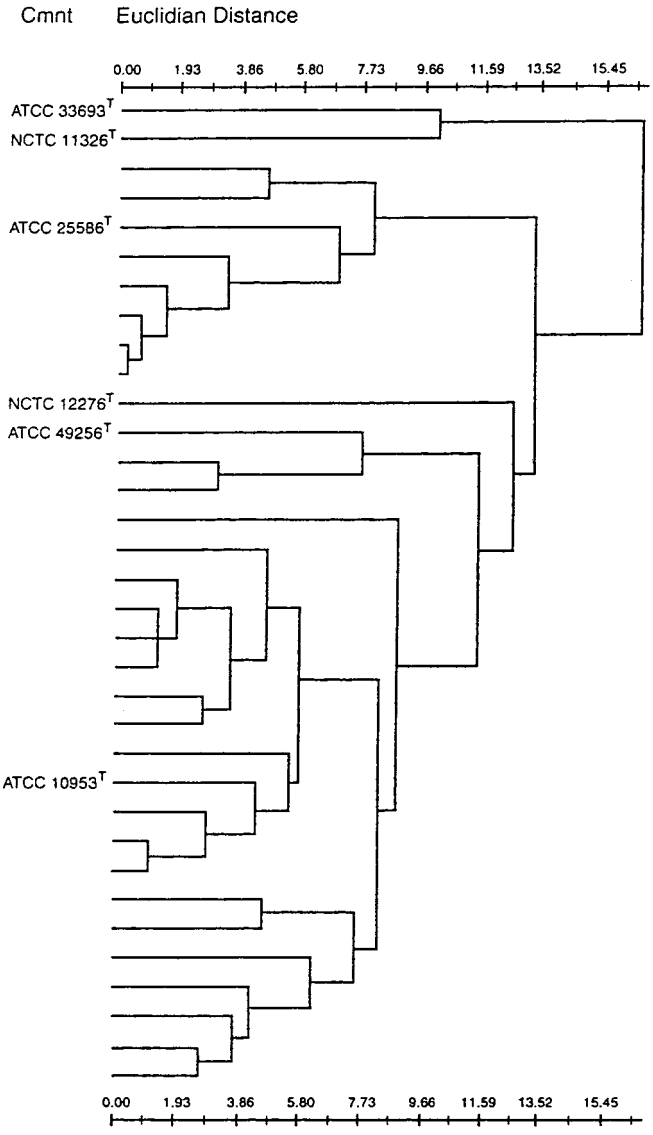

FIG. 1. Dendrogram of β-lactamase-producing *F. nucleatum* isolates and the type strains *F. nucleatum* subsp. *polymorphum* ATCC 10953^T, *F. nucleatum* subsp. *nucleatum* ATCC 25586^T, *F. nucleatum* subsp. *vincentii* ATCC 49256^T, *F. nucleatum* subsp. *fusiforme* NCTC 11326^T, *F. nucleatum* subsp. *animalis* NCTC 12276^T, and *F. periodonticum* ATCC 33693^T, generated by cluster analysis of CFA profiles.

well as present results, FAA favors the growth of fusobacteria and, conceivably, promotes the susceptibility testing of *F. nucleatum*.

In a recent study (14), the penicillin breakpoint of 0.5 μg/ml precisely separated β-lactamase-producing *Prevotella melaninogenica* isolates from non-β-lactamase-producing isolates; the observation is in accordance with the latest breakpoint determination by NCCLS (18). In the present study, the lowest MIC measured on FAA was 1 μg/ml for β-lactamase-producing *F. nucleatum*, but the MICs for two β-lactamase-negative *F. nucleatum* isolates from one child (probably the same strain) were also 1 μg/ml. The MICs of amoxicillin-clavulanic acid, tetracycline hydrochloride, metronidazole, and trovafloxacin were unambiguously below the current susceptible breakpoints approved by NCCLS (18). Trovafloxacin, a novel fluoroquinolone, has shown promising in vitro activity against anaerobes (25, 26). In the present study, the MICs of trovafloxacin and also of metronidazole were similar to MICs for 28 *F. nucleatum* strains reported by Wexler et al. (26). Although no NCCLS breakpoint for azithromycin has been approved for anaerobes, the low MICs seen in this study indicate that azithromycin has better activity than other macrolides against *F. nucleatum*.

The clinical significance of fusobacteria in pediatric infections has recently been pointed out by Brook (5). In fact, *F. nucleatum* was the *Fusobacterium* species most frequently isolated from these infections. According to our preliminary experience, *F. nucleatum* might play a role in the pathogenesis of acute otitis media in infancy, as it was the anaerobic species most frequently isolated from the nasopharynx during bouts of ear infection (12). As seen in the present study, penicillin resistance due to β-lactamase production by oral *F. nucleatum* occurs frequently in childhood. This phenomenon is not confined to *F. nucleatum* subsp. *polymorphum* but seems to be a characteristic of several other *F. nucleatum* subspecies. As beta-lactams are among the antimicrobials most commonly used in bacterial pediatric infections, routine β-lactamase testing of multiple isolates from such infections could be of benefit.

In conclusion, the reported high resistance rates among oral anaerobic species in childhood can have a significant impact on the treatment practices and outcomes of pediatric infections that originate in the oral cavity.

This study was supported in part by grants (E. Könönen) from the Finnish Cultural Foundation and the Finnish Dental Society.

The technical assistance of Marja Piekkola is gratefully acknowledged.

REFERENCES

1. **Aldridge, K. E., C. V. Sanders, A. C. Lewis, and R. L. Marier.** 1983. Susceptibility of anaerobic bacteria to beta-lactam antibiotics and beta-lactamase production. J. Med. Microbiol. **16:**75–82.
2. **Appelbaum, P. C., S. K. Spangler, and M. R. Jacobs.** 1990. β-Lactamase production and susceptibilities to amoxicillin, amoxicillin-clavulanate, ticarcillin, ticarcillin-clavulanate, cefoxitin, imipenem, and metronidazole of 320 non-*Bacteroides fragilis Bacteroides* isolates and 129 fusobacteria from 28 U.S. centers. Antimicrob. Agents Chemother. **34:**1546–1550.
3. **Bourgault, A.-M., G. K. Harding, J. A. Smith, G. B. Horsman, T. J. Marrie, and F. Lamothe.** 1986. Survey of anaerobic susceptibility patterns in Canada. Antimicrob. Agents Chemother. **30:**798–801.
4. **Brazier, J. S., E. J. C. Goldstein, D. M. Citron, and M. I. Ostovari.** 1990. Fastidious anaerobe agar compared with Wilkins-Chalgren agar, brain heart infusion agar, and brucella agar for susceptibility testing of *Fusobacterium* species. Antimicrob. Agents Chemother. **34:**2280–2282.
5. **Brook, I.** 1994. Fusobacterial infections in children. J. Infect. **28:**155–165.
6. **Dzink, J. L., M. T. Sheenan, and S. S. Socransky.** 1990. Proposal of three subspecies of *Fusobacterium nucleatum* Knorr 1922: *Fusobacterium nucleatum* subsp. *nucleatum* subsp. nov., comb. nov.; *Fusobacterium nucleatum* subsp. *polymorphum* subsp. nov., nom. rev., comb. nov.; and *Fusobacterium nucleatum* subsp. *vincentii* subsp. nov., nom. rev., comb. nov. Int. J. Syst. Bacteriol. **40:**74–78.
7. **Gharbia, S. E., and H. N. Shah.** 1992. *Fusobacterium nucleatum* subsp. *fusiforme* subsp. nov. and *Fusobacterium nucleatum* subsp. *animalis* subsp. nov. as additional subspecies within *Fusobacterium nucleatum*. Int. J. Syst. Bacteriol. **42:**296–298.
8. **Gharbia, S. E., H. N. Shah, P. A. Lawson, and M. Haapasalo.** 1990. The distribution and frequency of *Fusobacterium nucleatum* subspecies in the human oral cavity. Oral Microbiol. Immunol. **5:**324–327.
9. **Goldstein, E. J. C., P. H. Summanen, D. M. Citron, M. H. Rosove, and S. M. Finegold.** 1995. Fatal sepsis due to a β-lactamase-producing strain of *Fusobacterium nucleatum* subspecies *polymorphum*. Clin. Infect. Dis. **20:**797–800.
10. **Johnson, C. C., H. M. Wexler, S. Becker, M. Garcia, and S. M. Finegold.** 1989. Cell-wall-defective variants of *Fusobacterium*. Antimicrob. Agents Chemother. **33:**369–372.
11. **Könönen, E., S. Asikainen, M. Saarela, J. Karjalainen, and H. Jousimies-Somer.** 1994. The oral gram-negative anaerobic microflora in young children: longitudinal changes from edentulous to dentate mouth. Oral Microbiol. Immunol. **9:**136–141.
12. **Könönen, E., A. Kanervo, A. Bryk, A. Takala, R. Syrjänen, and H. Jousimies-Somer.** Anaerobes in the nasopharynx during acute otitis media episodes in infancy. Anaerobe, in press.
13. **Könönen, E., S. Nyfors, J. Mättö, S. Asikainen, and H. Jousimies-Somer.** 1997. β-Lactamase production by oral pigmented *Prevotella* species isolated from young children. Clin. Infect. Dis. **25**(Suppl. 2)**:**S272–S274.
14. **Könönen, E., M. Saarela, A. Kanervo, J. Karjalainen, S. Asikainen, and H. Jousimies-Somer.** 1995. β-Lactamase production and penicillin susceptibility among different ribotypes of *Prevotella melaninogenica* simultaneously colonizing the oral cavity. Clin. Infect. Dis. **20**(Suppl. 2)**:**S364–S366.
15. **Könönen, E., M.-L. Väisänen, S. M. Finegold, R. Heine, and H. Jousimies-Somer.** 1996. Cellular fatty acid analysis and enzyme profiles of *Porphyromonas catoniae*—a frequent colonizer of the oral cavity in children. Anaerobe **2:** 329–335.
16. **Moore, L. V. H., D. M. Bourne, and W. E. C. Moore.** 1994. Comparative distribution and taxonomic value of cellular fatty acids in thirty-three genera of anaerobic gram-negative bacilli. Int. J. Syst. Bacteriol. **44:**338–347.
17. **Morris, M. L., R. H. Andrews, and A. H. Rogers.** 1997. Investigations of the taxonomy and systematics of *Fusobacterium nucleatum* using allozyme electrophoresis. Int. J. Syst. Bacteriol. **47:**103–110.
18. **National Committee for Clinical Laboratory Standards.** 1997. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 4th ed. Approved standard M11-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
19. **Nyfors, S., E. Könönen, A. Takala, and H. Jousimies-Somer.** β-Lactamase production by oral gram-negative anaerobes during the first year of infant's life in relation to antibiotic treatment. Submitted for publication.
20. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1:**283–288.
21. **Summanen, P., E. J. Baron, D. M. Citron, C. A. Strong, H. M. Wexler, and S. M. Finegold.** 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing, Belmont, Calif.
22. **Summanen, P., H. M. Wexler, and S. M. Finegold.** 1992. Antimicrobial susceptibility testing of *Bilophila wadsworthia* by using triphenyltetrazolium chloride to facilitate endpoint determination. Antimicrob. Agents Chemother. **36:**1658–1664.
23. **Tunér, K., E. J. Baron, P. Summanen, and S. M. Finegold.** 1992. Cellular fatty acids in *Fusobacterium* species as a tool for identification. J. Clin. Microbiol. **30:**3225–3229.
24. **Tunér, K., L. Lindquist, and C. E. Nord.** 1985. Characterization of a new β-lactamase from *Fusobacterium nucleatum* by substrate profiles and chromatofocusing patterns. J. Antimicrob. Chemother. **16:**23–30.
25. **Väisänen, M.-L., J. Mättö, K. Salminen, and H. Jousimies-Somer.** 1997. In vitro activity of trovafloxacin against anaerobic bacteria. Rev. Med. Microbiol. **8**(Suppl. 1)**:**S81–S83.
26. **Wexler, H. M., E. Molitoris, D. Molitoris, and S. M. Finegold.** 1996. In vitro activities of trovafloxacin against 557 strains of anaerobic bacteria. Antimicrob. Agents Chemother. **40:**2232–2235.
27. **Xie, H., R. J. Gibbons, and D. I. Hay.** 1991. Adhesive properties of strains of *Fusobacterium nucleatum* of the subspecies *nucleatum*, *vincentii* and *polymorphum*. Oral Microbiol. Immunol. **6:**257–263.